DIPLOID AND TETRAPLOID *ARANDA* WENDY SCOTT FROM MERISTEM CULTURE

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ABSTRACT

Diploid and tetraploid plants arising from mericloning diploid Aranda Wendy Scott 'Greenfield' was investigated. Out of 77 mericloned plants 28 (36.4%) were tetraploid. Diploid plants grew slightly faster than tetraploid plants, produced more flower sprays, more flowers per spray, and longer sprays with longer scapes. The vase life was found to be slightly longer for the tetraploid sprays than the diploid sprays. Tetraploid flower parts were slightly larger than diploid flower parts.

Spray yield, spray length and number of flowers per spray are important in commercial cut flower production. Since the diploid form of Aranda Wendy Scott 'Greenfield' is superior to the tetraploid form, the tetraploid plants arising from meristem culture must be considered undesirable mutants for cut flower production.

The somatic chromosome number was found to be 38 for the diploid and 76 for the tetraploid. The diploid showed poor pairing of parental chromosomes at meiosis, and poor fertility. The tetraploid or amphidiploid exhibited a high degree of chromosome homology and the restoration of fertility. The polyploid mutation in Aranda Wendy Scott is undesirable for cut flower purposes, but might be useful for breeding.

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I. INTRODUCTION

The subtribe Vandinae is a large and diverse group in the family Orchidaceae. This subtribe includes the genera Vanda and Arachnis which are native to the Old World tropics and subtropics. Their distinctive features are monopodial growth and the absence of pseudobulbs and rhizomes. Species in Vanda and Arachnis can be successfully intercrossed.

Aranda Wendy Scott is an intergeneric hybrid of Vanda Rothschildiana (V. sanderiana x V. coerulea) and Arachnis hookeriana. The cultivar Aranda Wendy Scott 'Greenfield' has become an important cut flower orchid in Singapore. This cultivar was vegetatively propagated from cuttings over the past two decades to produce the quantity of plants now under commercial production in Singapore and neighboring countries. This method of clonal propagation, however, may be inadequate for rapid expansion of production. The orchid meristem culture technique developed by Morel in 1960 has been applied to the rapid clonal increase of many types of orchids.

Cheah and Sagawa (1978) successfully mericloned Aranda Wendy Scott 'Greenfield'. Preliminary observation of the mericloned plants revealed variations in vegetative and floral characteristics associated with a change in chromosome number. The purpose of this investigation was to determine the degree of variation in the population and to compare morphological and cytological characteristics in diploid and tetraploid forms derived from meristem culture.

II. REVIEW OF LITERATURE

Vanda is a genus of 30 to 40 monopodial, epiphytic orchid species indigenous to the Philippines, East Indies and the Indo-Malayan region of Southeast Asia (Holttum, 1953). It is classified into the following groups according to type of leaf:

1) terete - cylindrical leaves, circular in cross-section,
2) semi-terete - more or less half-cylindrical leaves, crescent shaped in cross section,
3) strap or spatulate - more or less flat bladed, strap-like, two halves of the blades fold in toward each other.

The genus Arachnis comprises about 15 species that are distributed in the Malay Peninsula, Borneo, Sumatra and neighboring islands (Holttum, 1953). Arachnis hookeriana (White Scorpion Orchid) is cultivated as a cut flower because of its floriferousness and desirable size of the inflorescences. The flowers are creamy white to yellowish with a fine purple mottling (Holttum, 1967).

Aranda Wendy Scott is a hybrid of strap-leaved Vanda Rothschildiana (Vanda sanderiana x V. coerulea) and Arachnis hookeriana produced by J. L. Pestana in Singapore in 1956 (Sander and Wreford, 1961). Presently it is an important cut flower orchid of Singapore.

The orchid meristem culture technique developed by Morel in 1960 has been successfully applied for rapid clonal propagation of several commercially important orchids such as cattleya (Reinert and Mohr, 1967; Scully, 1967; Kim et al., 1970), and vanda (Kunisaki et al., 1972; Teo et al., 1973). More recently, Cheah and Sagawa (1978) successfully mericloned Aranda Wendy Scott.

Since meristem culture or shoot-tip culture is a method of clonal propagation, the resulting plants or mericlones are expected to be genetically identical to the original plant from which explants are obtained (Khaw and Ong, 1974-75; Vajrabhaya, 1977). However, mutations have been known to arise in orchid meristem culture (Bertsch, 1967; Khaw and Ong, 1974-75; Vajrabhaya and Vajrabhaya, 1974; Vajrabhaya, 1977). The environment in vitro (highly different from the environment in vivo) has a considerable effect on the selection of new cell types in a tissue. Mutations are always possible and the selection of new cell types during tissue culture leads to the production of new genotypes and phenotypes in vitro (Vajrabhaya, 1977). Tissue culture cells will continue to divide and not only express changes in the genetic material, but also perpetuate these selected cells (Partanen, 1965). With repeated subculturing of the same orchid tissue, more variations are likely to occur. When clones are propagated by tissue culture and when the population gets very large, more variants are expected. Also, the artificial environment during the tissue culture process may produce variants which normally would not appear (Vajrabhaya, 1977).

One type of chromosomal variation is polyploidy which involves the increase in genetic material by whole sets of the chromosome complement. Somatic polyploidy results when the coupling of chromosome replication and mitosis is loosened and the function of the spindle apparatus is disturbed (D'Amato, 1965).

Variants produced by meristem culture may be a valuable source of new plant material if they show improvement in characters, such as flower quality and yield. Once these desirable mutants are found, they can be asexually propagated to maintain their quality (Vajrabhaya,

1977). Selected variants can be propagated and utilized in a breeding program for possible improvement (Vahrabhaya and Vajrabhaya, 1974).

The most universal effect of polyploidy is an increase in cell size. This does not necessarily increase plant size as a whole since a common effect of polyploidy is a reduction in cell division during plant development. 'Gigas' effects of polyploidy are commonly found, particularly in organs such as flowers and leaves which have a determinate pattern of growth; leaves and petals of polyploids are usually thicker and firmer than their diploid counterparts (Stebbins, 1971). In orchids, tetraploid plants produce larger flowers with increased fertility and larger stomata, but generally the vegetative growth is not very different from the diploid plants (Vajrabhaya, 1977).

According to Wimber and Wimber (1968), tetraploid cymbidiums generally show an improvement in flower shape through an increase in width relative to the length of the floral parts, but they usually exhibit less vigor in plant growth and productivity. Investigations by Leonhardt (1977) revealed an increase in "fullness" and less space between floral parts, and the average number of flowers per inflorescence length was slightly reduced in the tetraploid form of Cymbidium Peter Pan 'Greensleeves'. Vajrabhaya (1977) claims that when orchids are grown for cut flower purposes, the number of flowers is a major desirable factor and that diploids have a greater productivity than tetraploids.

The most important factor which indicates the relationship between 2 parents is the chromosome pairing of the hybrid, known as homology (Stebbins, 1971). Tanaka and Kamemoto (1961) studied the meiotic chromosome behavior of the cross Arachnis hookeriana x Vanda suavis

and concluded that this hybrid had a low level of homology of parental chromosomes.

Species belonging to the same group (terete, semi-terete, or strap) in Vanda produce hybrids which tend to be fertile due to the close homology of the parental chromosomes (Storey, 1955). Tanaka and Kamemoto (1960) found V. luzonica x V. sanderiana (a hybrid between 2 strap species) to have a high degree of homology of the chromosomes.

If a diploid hybrid is infertile due to irregular meiosis, doubling the chromosomes would provide 2 sets of chromosomes that are alike. This may increase fertility by increasing meiotic homology (Stebbins, 1971). Kamemoto, et al. (1964) found a low level of homology of the meiotic chromosomes in the diploid hybrid Dendrobium Jaquelyn Thomas, an intersectional hybrid between D. phalaenopsis from the Phalaenanthe section and D. gouldii from the Ceratobium section. However, by doubling the chromosome number of this hybrid from 38 to 76, complete regularity was obtained and fertility was restored.

III. MATERIALS AND METHODS

Two groups of mericlones of Aranda Wendy Scott 'Greenfield' grown in 2-inch pots were received from Dr. Y. Sagawa. Group I consisted of 38 plants which was received on June 13, 1977. Group II consisted of 39 plants received on January 13, 1978. These plants were potted into 6-inch pots in crushed rock and grown in 30% saran shadehouse at the Mauka Campus of the University of Hawaii at Manoa. Plants were watered 3 times a week. Gaviota Foliar 60 fertilizer (20-20-20) was applied at a biweekly rate of ½ lb/100 gallons.

A completely randomized design was employed. The height of plants was measured from the base of the shoot to the base of the crotch of the top 2 leaves (rounding off to the nearest half centimeter). Measurements were taken at 3 to 4-month intervals. Flower sprays (racemes) were harvested when all buds on the spray were fully open. Sprays were placed in tap water in 500 ml Erlenmeyer flasks and kept in an air-conditioned laboratory at about 25°C. The vase life expressed as half life was determined by the number of days required for half of the total number of flowers on a spray to wilt, dry or drop. The water was changed every 5-6 days.

Scape length of each spray was measured to the nearest half centimeter as the distance from the base of the peduncle to the tip of the spray. The number of flowers per spray was also recorded.

Flower size was established as the length of the flower (dorsal sepal to lateral sepal) and the width of the flower (petal to petal) to the nearest millimeter of the third lowest flower on the spray. The lengths and widths of component flower parts (except the labellum) were also measured to the nearest millimeter (Fig. 7).

Somatic chromosome counts were determined for each plant. Actively growing root tips about 1-2 millimeters in length were placed in a small vial containing 0.002 M 8-hydroxyquinoline solution for 3-5 hours at about 18°C, and rinsed with deionized water. The root tips were then fixed in a modified Carnoy's solution (1:1:2 mixture of 95% ethyl alcohol, chloroform and glacial acetic acid) for 20 minutes at 10°C, hydrolyzed in 1 N hydrochloric acid for 8-10 minutes at 50° C, rinsed in deionized water and kept in 45% acetic acid for 10 minutes. The root cap was removed from the root tip in a drop of 45% acetic acid on a clean microslide with the use of dissecting needles under a dissecting microscope. The remaining tissue was cut into small pieces, and one drop of 1% aceto-orcein stain was added. The microslide was then placed in a plastic chamber saturated with 45% acetic acid for 8-10 minutes. A coverslip was applied for squashing, and air bubbles and excess stain were removed by applying pressure evenly and firmly on the coverslip after the slide was slightly heated. The coverslip was sealed with melted dental wax. The prepared squashes were examined under a light microscope, and chromosome numbers were determined. Photomicrographs of selected cells at metaphase of the mitotic cycle were taken with a Leitz microscope with a 35 mm Zeiss camera on black and white Kodak High Contrast Copy film.

Meiotic studies were made utilizing the pollinia of young flower buds. Buds were harvested from the sprays and placed on wet filter paper in a glass petri dish. A small portion of the pollinium was excised from the bud, cut into small pieces and placed in a drop of 45% acetic acid at 16° C on a microslide for 15 minutes in a plastic chamber saturated with 45% acetic acid. Excess 45% acetic acid was

blotted with bibulous paper, and a drop of 1% aceto-orcein stain was added for another 8-10 minutes in the plastic chamber. A coverslip was placed over the material, pressed firmly after gentle heating, then sealed with melted dental wax.

The prepared slides were examined under a light microscope to determine the stage of meiotic cell division of the pollen mother cells (PMCs). If the meiotic stage was before Metaphase I, the flower bud was carefully sealed and placed on wet filter paper in a petri dish and subsequently observed for Metaphase I. The pairing of homologous chromosomes at Metaphase I was analyzed. All other stages of meiosis were also observed. Photomicrographs of selected meiotic stages and sporad formation were taken.

To determine the fertility of Aranda Wendy Scott 'Greenfield', diploid and tetraploid individuals were selfed and crossed with diploid and tetraploid forms of Vanda Miss Joaquim, and diploid forms of V. lamellata and V. sanderiana. Fruits were harvested after about 3 months. The seeds were germinated in 125 ml Erlenmeyer flasks containing about 25 ml of modified Vacin and Went medium. The percentage of viable seeds was determined by examining 100 seeds from each fruit. Seeds were spread on a microscope slide with 1% aceto-orcein stain and examined under low power (100X) of a light microscope.

IV. RESULTS

The frequencies of diploid and tetraploid plants of Aranda Wendy Scott 'Greenfield' in the two groups of mericlones are shown in Table I. The percentage of tetraploids was much higher in Group I (55.3%) than in Group II (17.9%).

In Group I the rate of vegetative growth between the diploid and tetraploid plants was not significant at the 5% level. However, there was a significant difference at the 10% level, suggesting that the diploids grew at a slightly faster rate than the tetraploids (Table 2 and Fig. 1). In Group II, the difference in rate of vegetative growth between diploids and tetraploids was significant at the 5% level (Table 3 and Fig. 2), with the diploids again growing faster than the tetraploids.

The diploids were more productive than the tetraploids in both Group I and Group II (Tables 2 and 3, respectively). The average yield per plant of the diploids in Group I was higher than that of the tetraploids during most of the 2-month intervals over a period of two years (Fig. 3). The diploids in Group II also showed a higher yield per plant over a 16-month period, but the difference was not significant. There were only 7 tetraploid plants in this group, only 3 of which produced any flower sprays. Consequently, Fig. 4 reflects several 2-month periods without any yields.

The comparison between diploid and tetraploid flower sprays is shown in Figure 5. Diploid plants in Group I produced flower sprays with significantly more flowers per spray which was correlated with longer scapes and longer sprays than the tetraploids. The diploids in Group II also had longer scapes and longer sprays than the tetraploids.

Table 1. -- Frequency of diploid and tetraploid plants from mericloning diploid Aranda Wendy Scott 'Greenfield'.

Group	2N	4N	Total	% 4N
I	17	21	38	55.3
II	32	7	39	17.9
Total	49	28	77	36.4

Table 2. -- Plant growth, spray yield, spray characters, and half life of diploid and tetraploid Aranda Wendy Scott 'Greenfield' (Group I).

Character	Confidence Interval of Character Measurement		Significant Difference at $t_{.05}$
	2N	4N	
Vegetative Growth (cm)	49.5 ± 2.47	45.0 ± 2.58	NS*
Total Spray Yield/Plant/2 Years	8.4 ± 1.48	5.0 ± 1.02	S
Number of Flowers/Spray	11.0 ± 0.29	9.4 ± 0.37	S
Scape Length (cm)	27.2 ± 0.59	21.9 ± 0.51	S
Spray Length (cm)	46.4 ± 1.05	34.6 ± 0.71	S
Half Life (days)	13.1 ± 0.65	14.7 ± 0.74	S

* The difference was significant at $t_{.10}$

Figure 1 Vegetative growth of diploid and tetraploid Aranda Wendy Scott 'Greenfield' (Group I - based on 17 diploid and 21 tetraploid plants).

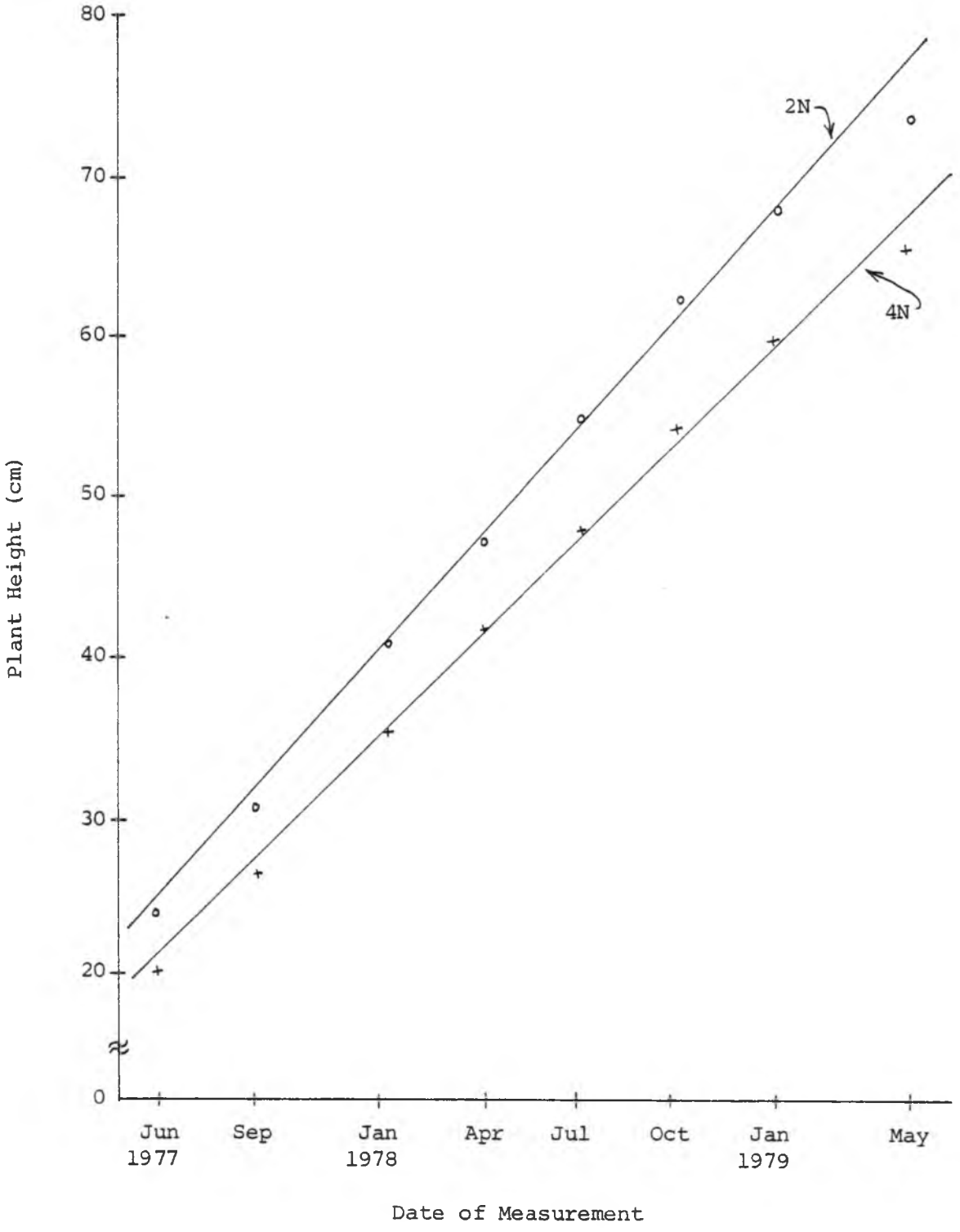

80
70
60
50
40
30
20
0
2N
4N
Plant Height (cm)
Jun
1977
Sep
Jan
1978
Apr
Jul
Oct
Jan
1979
May
Date of Measurement

Table 3. -- Plant growth, spray yield, spray characters, and half life of diploid and tetraploid Aranda Wendy Scott 'Greenfield' (Group II).

Character	Confidence Interval of Character Measurement		Significant Difference at $t_{.05}$
	2N	4N	
Vegetative Growth (cm)	30.3 ± 2.05	24.1 ± 3.43	S
Total Spray Yield/Plant/2 Years	3.4 ± 0.99	1.7 ± 1.43	NS
Number of Flowers/Spray	9.5 ± 0.52	7.6 ± 1.88	NS
Scape Length (cm)	25.6 ± 0.94	18.6 ± 3.86	S
Spray Length (cm)	42.0 ± 1.68	29.0 ± 6.32	S
Half Life (days)	13.1 ± 0.91	15.2 ± 4.92	NS

Figure 2. Vegetative growth of diploid and tetraploid Aranda Wendy Scott 'Greenfield' (Group II - based on 32 diploid and 7 tetraploid plants).

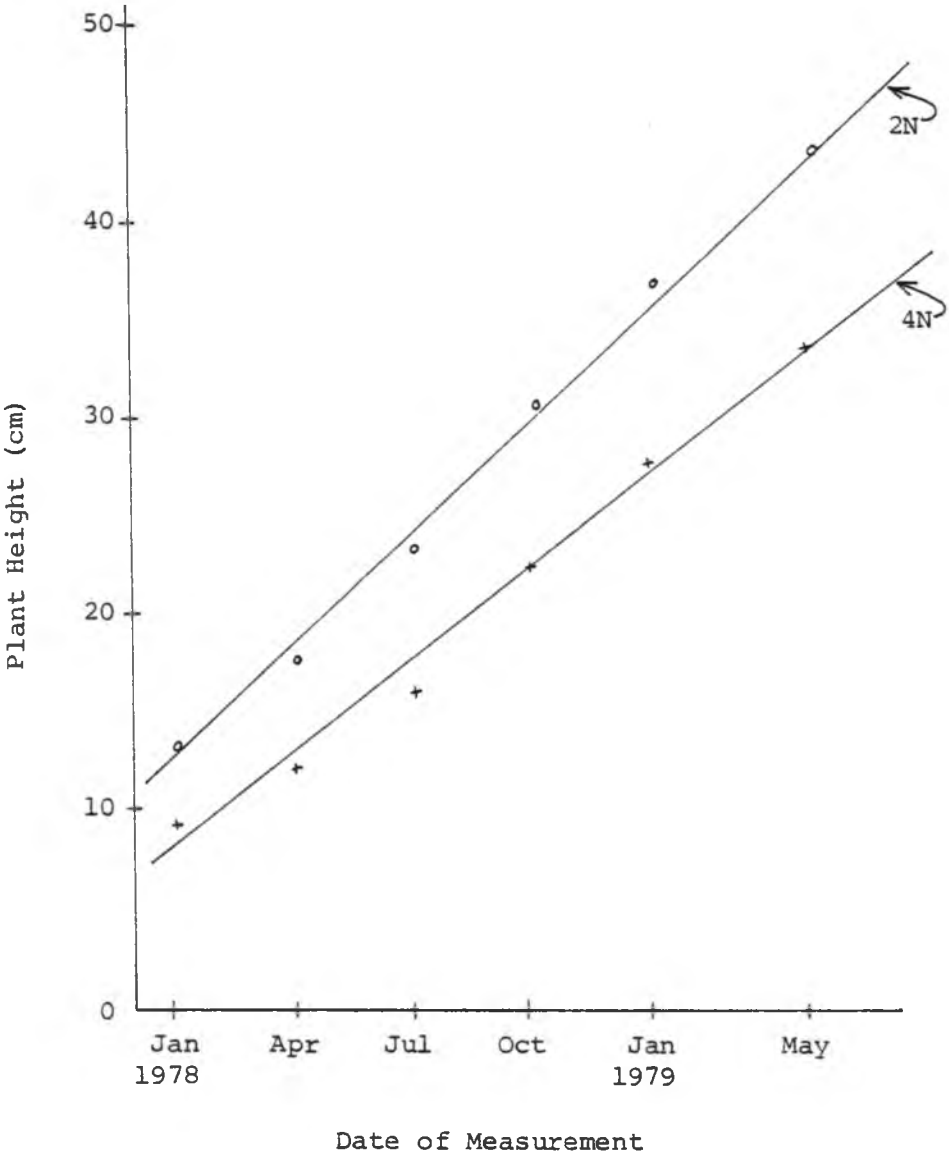

50
40
30
20
10
0
Plant Height (cm)
2N
4N
Jan
1978
Apr
Jul
Oct
Jan
1979
May
Date of Measurement

Figure 3. Bimonthly flower production for 24 months of diploid and tetraploid Aranda Wendy Scott 'Greenfield' (Group I - based on 17 diploid plants and 21 tetraploid plants).

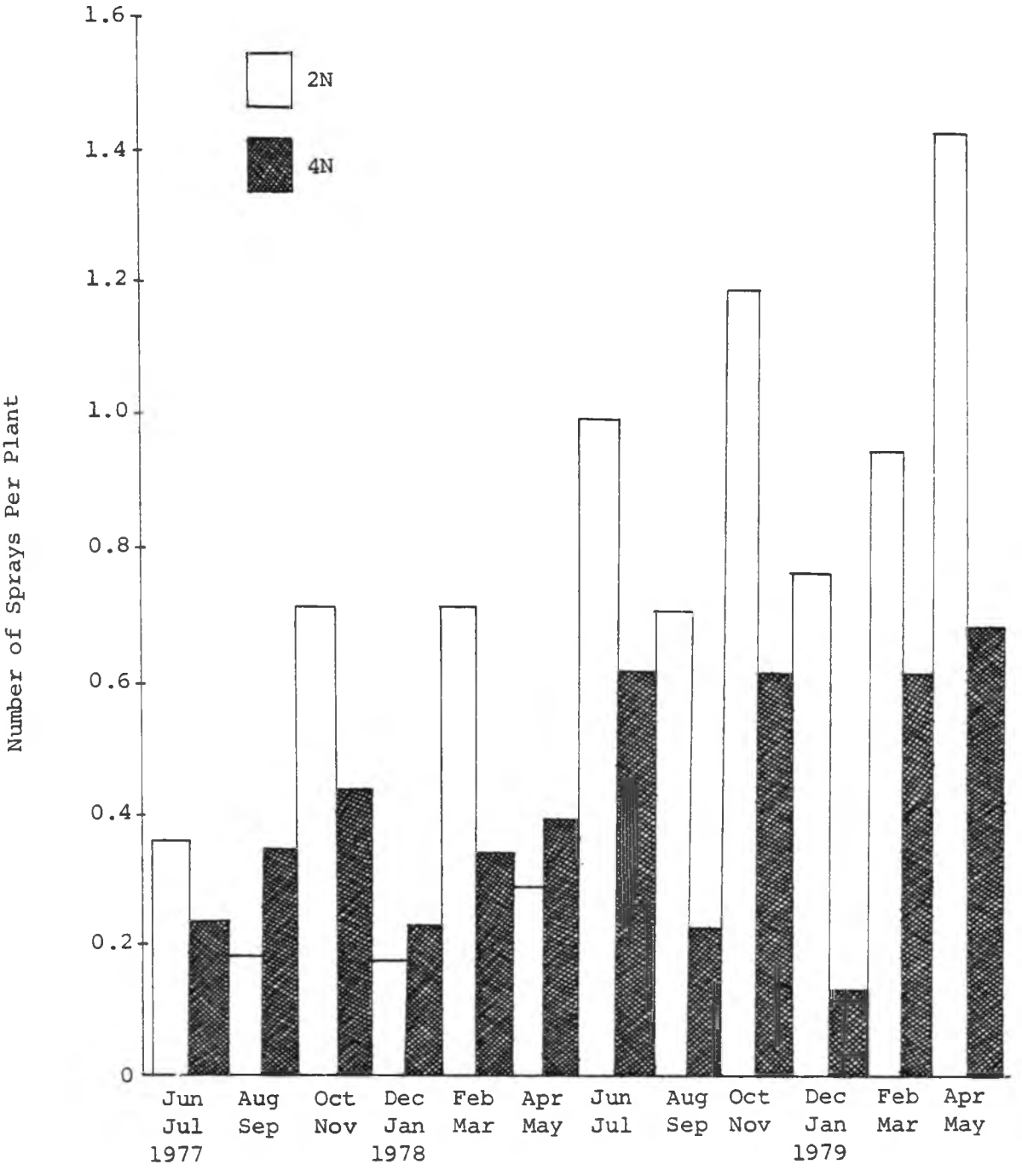
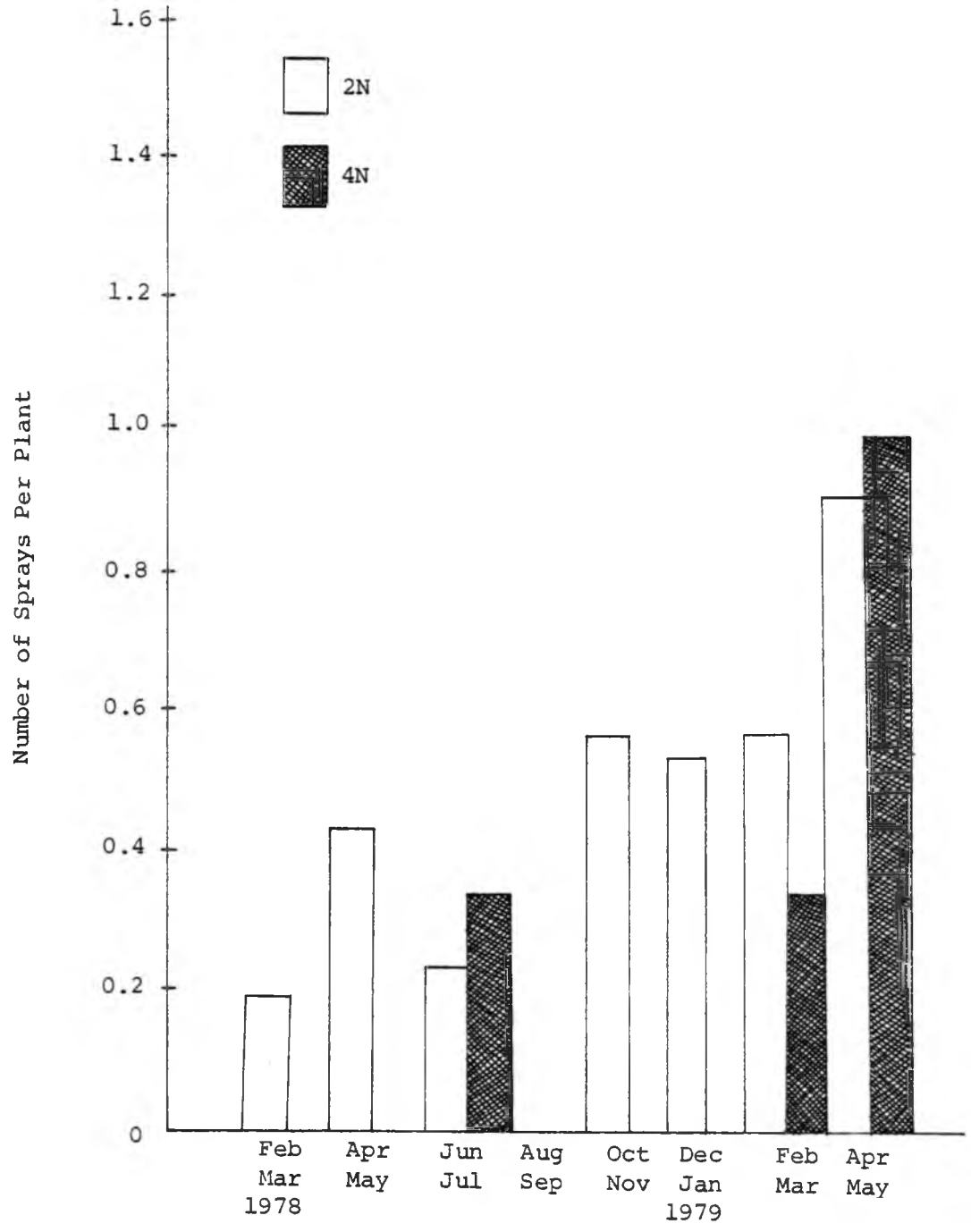

Number of Sprays Per Plant
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
2N
4N
Jun Jul
Aug Sep
Oct Nov
Dec Jan
Feb Mar
Apr May
Jun Jul
Aug Sep
Oct Nov
Dec Jan
Feb Mar
Apr May
1977
1978
1979

Figure 4. Bimonthly flower production for 16 months of diploid and tetraploid Aranda Wendy Scott 'Greenfield' (Group II - based on 21 diploid plants and 3 tetraploid plants).


Number of Sprays Per Plant
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
2N
4N
Feb
Mar
1978
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
1979
Feb
Mar
Apr
May

However, the number of flowers per spray was not significant. In Group I, tetraploid sprays lasted longer than diploid sprays but not in Group II. The inconsistency between Groups I and II is probably due to the difference in age of plants and the low number of sprays produced by the tetraploid plants in Group II (only 5 sprays produced by 3 plants during the 16-month period).

Tables 4 and 5 show the comparison in flower morphology between diploids and tetraploids in Groups I and II, respectively. Tetraploid flowers and their component flower parts were slightly larger than diploid flowers (Fig. 6) in Group I. Ploidy level did not influence size of flower parts in Group II.

Leonhardt (1977) developed a formula for testing the effects of polyploidy on floral morphology in diploid and tetraploid Cymbidium Peter Pan 'Greensleeves' for fullness:

$$\frac{\text{overall flower width}}{\frac{\text{dorsal sepal width} + \text{labellum width} + 2(\text{lateral sepal width} + \text{petal width})}{6}}$$

Since the labellum does not contribute much to the overall shape (Fig. 7) of Aranda Wendy Scott, the formula was modified to:

$$\frac{\frac{1}{2}(\text{flower width} + \text{flower length})}{\frac{\text{dorsal speal width} + 2(\text{lateral sepal width} + \text{petal width})}{5}}$$

where the numerator is the overall flower size, and the denominator is the average floral segment width. When the data from Group I (Table 4) are applied to the modified formula, the following is obtained:

diploid: $\dfrac{.5(7.9 + 9.5)}{.2[1.6 + 2(1.9 + 1.7)]} = 4.94$

tetraploid: $\dfrac{.5(8.3 + 10.2)}{.2[1.8 + 2(2.0 + 1.8)]} = 4.92$

Plate I. Flower sprays and individual flowers of diploid and tetraploid Aranda Wendy Scott 'Greenfield'.

Figure 5 Flower spray, left--diploid, right--tetraploid (0.2X).

Figure 6 Individual flowers, left--diploid, right-- tetraploid (0.5X).


5


6

Figure 7. Terminology of floral segments of an Aranda Wendy Scott flower.

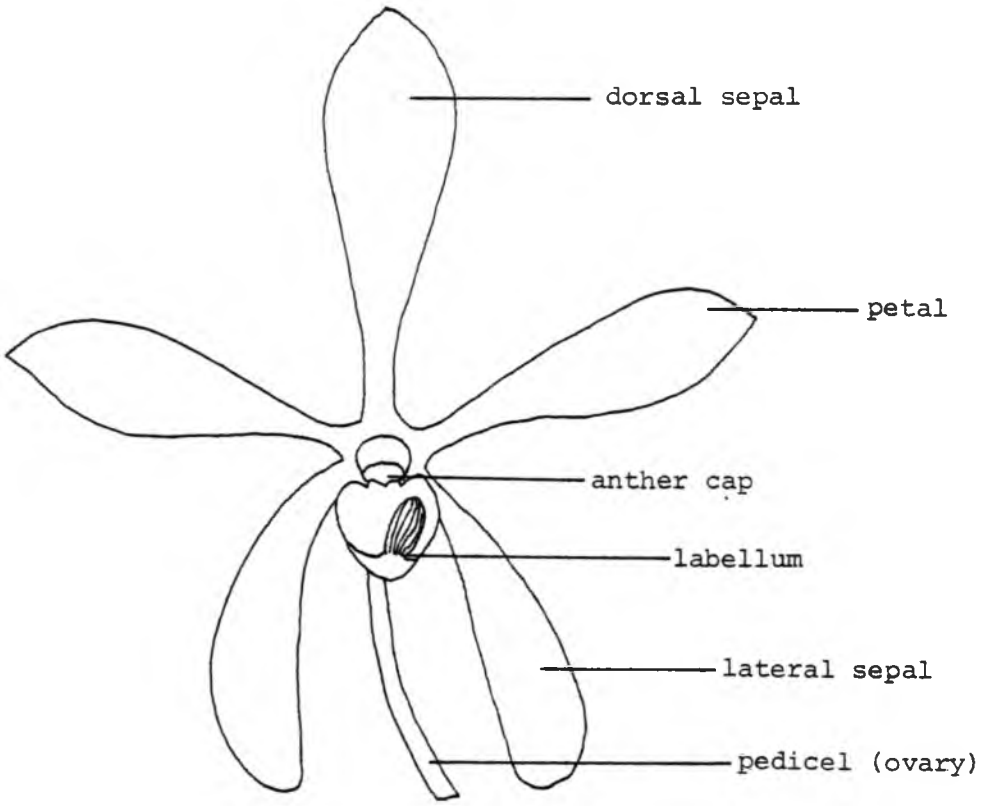

dorsal sepal
petal
anther cap
labellum
lateral sepal
pedicel (ovary)

Table 4. -- Flower morphology of diploid and tetraploid Aranda Wendy Scott 'Greenfield' (Group I).

Character	Confidence Interval of Character Measurement		Significant Difference at $t_{.05}$
	2N	4N	
	Size (cm)		
Pedicel and Ovary Length	4.7 ± 0.07	4.7 ± 0.07	NS
Flower Length	9.5 ± 0.08	10.2 ± 0.15	S
Flower Width	7.9 ± 0.10	8.3 ± 0.15	S
Petal Length	4.2 ± 0.03	4.5 ± 0.06	S
Petal Width	1.7 ± 0.02	1.8 ± 0.02	S
Dorsal Sepal Length	4.9 ± 0.07	5.2 ± 0.09	S
Dorsal Sepal Width	1.6 ± 0.02	1.8 ± 0.03	S
Lateral Sepal Length	4.5 ± 0.04	4.7 ± 0.06	S
Lateral Sepal Width	1.9 ± 0.02	2.0 ± 0.03	S

Table 5. -- Flower morphology of diploid and tetraploid Aranda Wendy Scott 'Greenfield' (Group II).

Character	Confidence Interval of Character Measurement		Significant Difference at $t_{.05}$
	2N	4N	
	Size (cm)		
Pedicel and Ovary Length	4.4 ± 0.09	4.4 ± 0.48	NS
Flower Length	9.3 ± 0.13	9.6 ± 0.79	NS
Flower Width	7.8 ± 0.13	8.2 ± 0.62	NS
Petal Length	4.1 ± 0.05	4.2 ± 0.25	NS
Petal Width	1.6 ± 0.02	1.8 ± 0.16	NS
Dorsal Sepal Length	4.8 ± 0.06	4.9 ± 0.36	NS
Dorsal Sepal Width	1.6 ± 0.02	1.7 ± 0.09	NS
Lateral Sepal Length	4.4 ± 0.05	4.4 ± 0.34	NS
Lateral Sepal Width	1.8 ± 0.03	1.9 ± 0.14	NS

There was no difference in the ratio of the overall flower size to the average floral segment width for the diploid and the tetraploid.

The chromosome number of the diploid Aranda Wendy Scott was 2n=2x=38 (Fig. 8), and the tetraploid 2n=4x=76 (Fig. 9). Meiosis in the diploid exhibited a wide range of univalents and bivalents at Metaphase I. Figure 10 represents a cell with 12 bivalents (II) and 14 univalents (I) with a corresponding drawing of the chromosomes in Figure 11. The number of bivalents ranged from 3 to 16, with a mean of 8.8 bivalents, and the most frequent configuration was 9 bivalents and 20 univalents (Table 6). Cells shown in Figures 12-14 reveal the highly irregular behavior of meiotic chromosomes. The genomes of Vanda and Arachnis in this hybrid exhibited a very low level of homology. At Anaphase I, univalents and bivalents often migrated at random to the poles or were unoriented and formed many nuclei. The sporads (Figures 15 and 16) ranged from dyands (2 spores) to nonads (9 spores) (Table 7). Dyads with or without microcytes occurred most frequently (35.3%).

Meiosis in tetraploid Aranda Wendy Scott was more regular than that of the diploid. Analysis of meiosis of tetraploids was diffcult due to the high number of chromosomes, and their clumpiness. Several stages of meiosis are shown in Figures 17-21. A total of 400 sporads were observed which reflected the degree of regularity of meiosis. In the tetraploid, nearly 100% were tetrads (4 spores) (Figures 22-24), and a very small percentage were triads (3 spores) (Table 8). Of the tetrads, 61.8% did not contain any microcytes.

Table 9 shows the crosses which were made with both diploid and tetraploid Aranda Wendy Scott 'Greenfield' to determine differences in fertility. All pods (fruits) of the crosses made with the diploid

Plate II. Chromosomes of diploid and tetraploid Aranda Wendy Scott.

Figure 8 Somatic chromosomes of diploid Aranda Wendy Scott, 2n=2x=38 (1650X).

Figure 9 Somatic chromosomes of tetraploid Aranda Wendy Scott, 2n=4x=76 (1650X).

Figure 10 Meiotic chromosomes at Metaphase I in a pollen mother cell (PMC) of diploid Aranda Wendy Scott, showing 12 II and 14 I (1650X).

Figure 11 Drawing of meiotic chromosomes in Fig.10, indicating the location of the bivalents (II).

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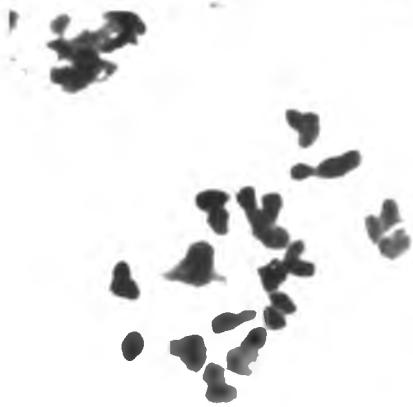

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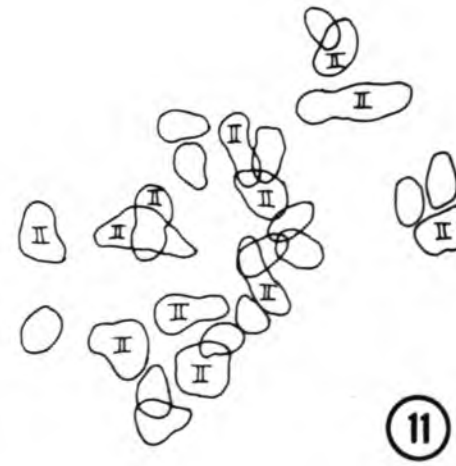

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Table 6. -- Frequencies of chromosome configurations at Metaphase I of the intergeneric diploid hybrid Aranda Wendy Scott 'Greenfield'

	Chromosome Configurations														
	16^{II} 6^{I}	15^{II} 8^{I}	14^{II} 10^{I}	13^{II} 12^{I}	12^{II} 14^{I}	11^{II} 16^{I}	10^{II} 18^{I}	9^{II} 20^{I}	8^{II} 22^{I}	7^{II} 24^{I}	6^{II} 26^{I}	5^{II} 28^{I}	4^{II} 30^{I}	3^{II} 32^{I}	
Number of Cells	1	0	2	2	1	2	1	6	1	2	2	1	1	3	25 Total Cells

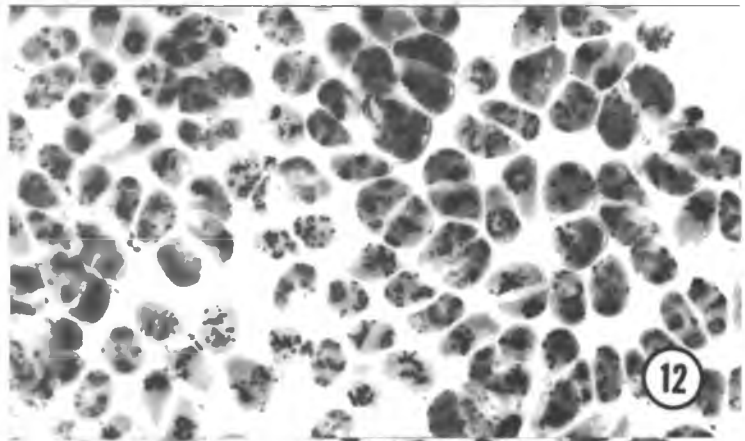
$\bar{X} = 8.8^{II}, 20.4^{I}$

Plate III. Irregular meiotic behavior in diploid Aranda Wendy Scott.

Figure 12 Field view of PMCs in various stages of meiosis showing highly unsynchronized behavior (235X).

Figure 13 Higher magnification of PMCs showing cells in Telophase I, early Prophase II, Metaphase II, and Telophase II (428X).

Figure 14 PMC in Telophase I (1650X).



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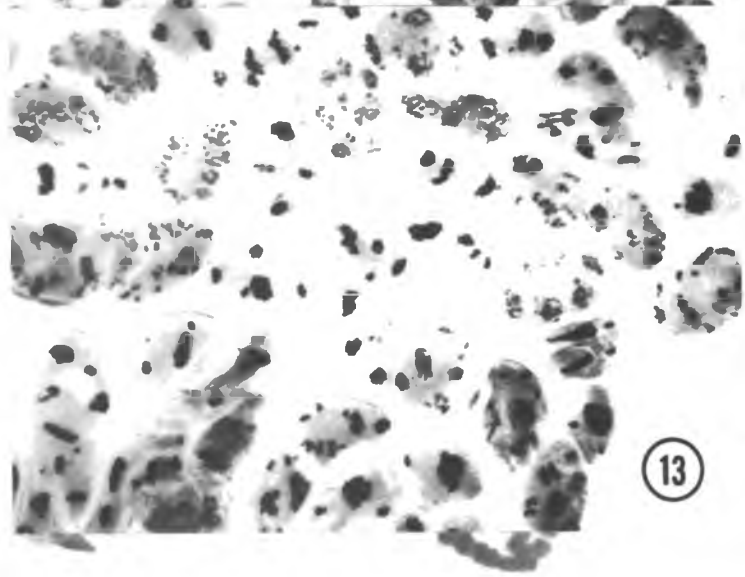

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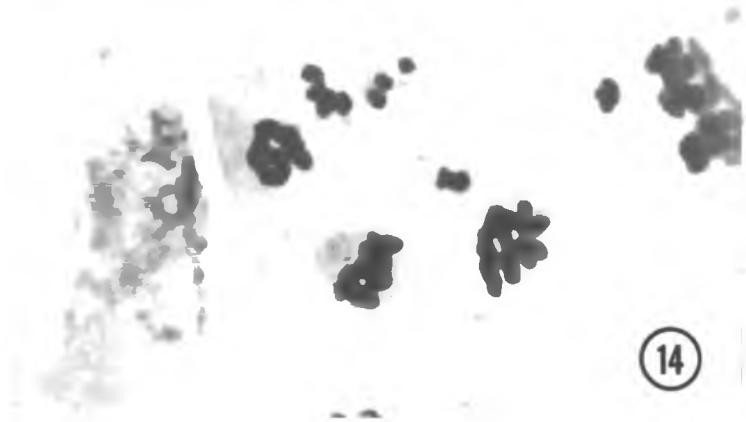

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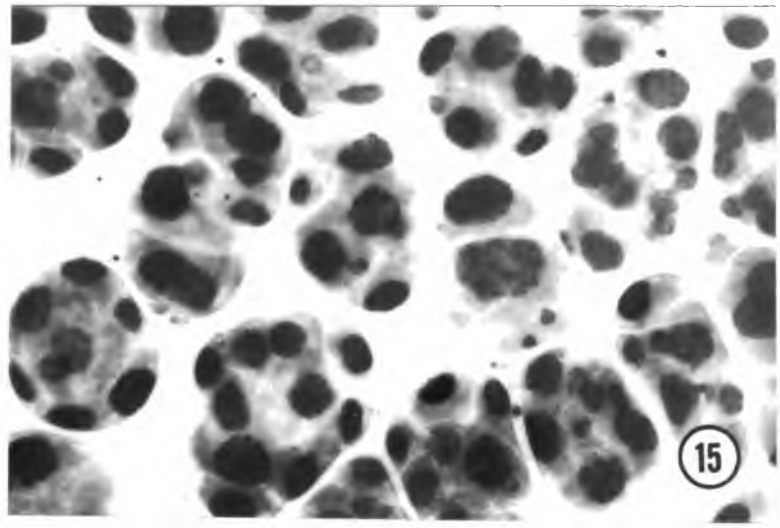
Plate IV. Sporad formation in diploid Aranda Wendy Scott.

Figure 15 Field view of a wide range of sporad types (note dyad with 2 microcytes) (667X).

Figure 16 Large dyad (2 spores) next to a hexad (6 spores) (1650X)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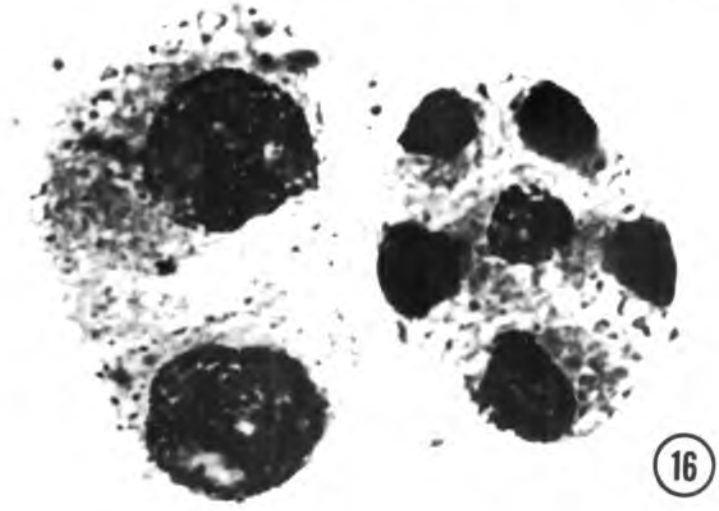
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Table 7. -- Sporad formation in diploid Aranda Wendy Scott 'Greenfield'.

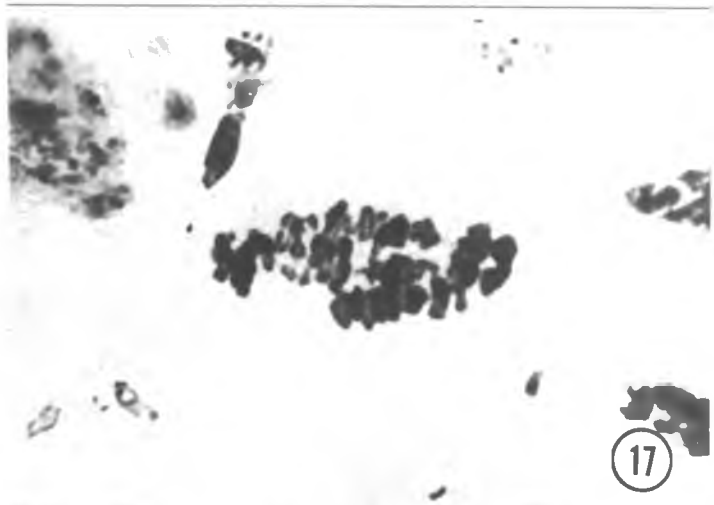
No. of micro-cytes	Dyad	%	Triad	%	Tetrad	%	Pentad	%	Hexad	%	Heptad	%	Octad	%	Nonad	%
0	9	3.0	1	0.3	18	6.0	13	4.3	19	6.3	11	3.7	7	2.3	4	1.3
1	9	3.0	2	0.7	14	4.7	13	4.3	11	3.7	11	3.7	1	0.3	1	0.3
2	27	9.0	1	0.3	16	5.3	4	1.3	3	1.0	2	0.7	1	0.3	2	0.7
3	14	4.7	3	1.0	13	4.3	2	0.7	2	0.7	2	0.7	1	0.3	1	0.3
4	16	5.3	0	0.0	3	1.0	0	0.0	2	0.7	1	0.3	0	0.0	1	0.3
5	17	5.7	0	0.0	4	1.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
6	7	2.3	0	0.0	0	0.0	0	0.0	0	0.0	1	0.3	0	0.0	0	0.0
7	5	1.7	1	0.3	0	0.0	0	0.0	1	0.3	0	0.0	0	0.0	0	0.0
8	2	0.7	0	0.0	0	0.0	0	0.0	1	0.3	0	0.0	0	0.0	0	0.0
Total No. of Sporads	106		8		68		32		39		28		10		9	Total of 300 Sporads
%		35.3		2.7		22.7		10.7		13.0		9.3			6.3	

Plate V. Metaphase I and Anaphase I of meiosis in tetraploid Aranda Wendy Scott.

Figure 17 Chromosomes at Metaphase I (1650X).

Figure 18 Homologous chromosomes separate and migrate to opposite poles at Anaphase I (1650X).

Figure 19 Separation of homologous chromosome complete as they migrate further to opposite poles (pre-Telophase I) (1650X).



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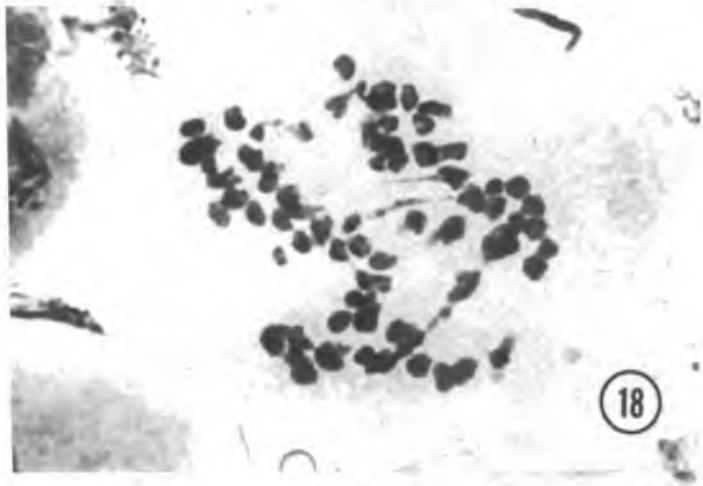

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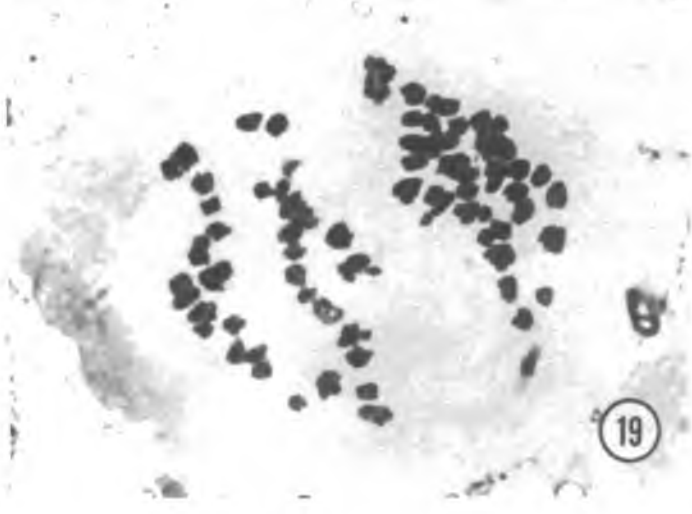

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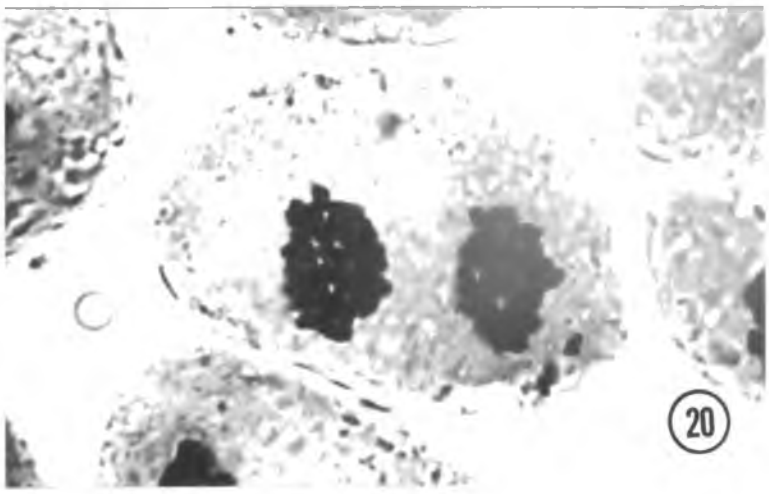
Plate VI. Two meiotic stages in tetraploid Aranda Wendy Scott.

Figure 20 Telophase I, two groups of chromosomes (1650X).

Figure 21 Metaphase of microspore division showing linear arrangement (note the 2 stray chromosomes which will form a microcyte) (1650X).



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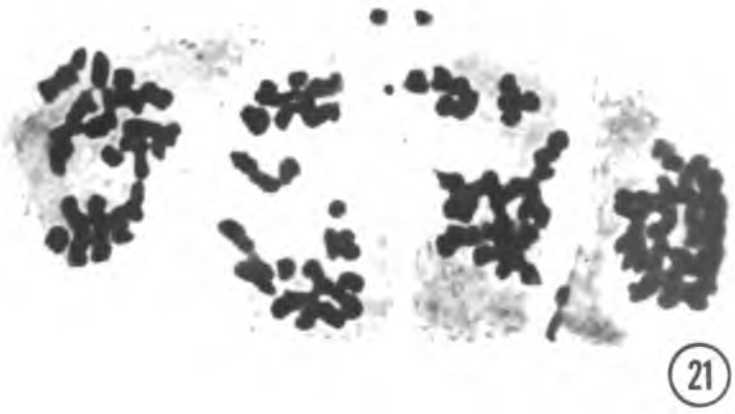

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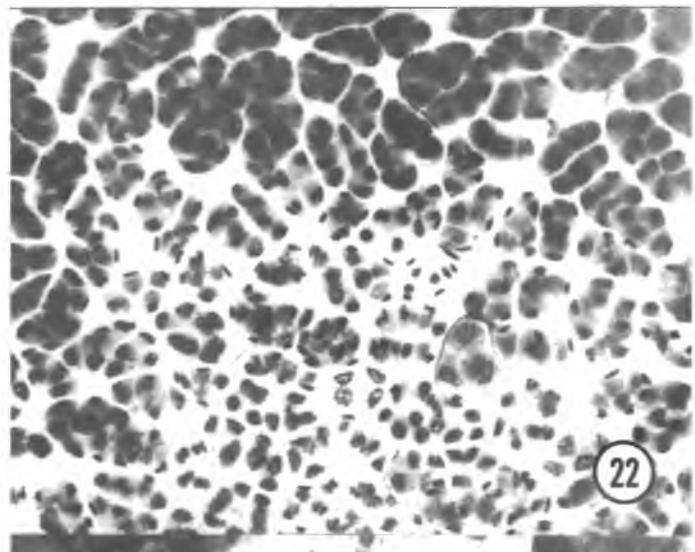
Plate VII. Sporad formation in tetraploid Aranda Wendy Scott.

Figure 22 Field view of sporads showing a few cells in Telophase II (216X).

Figure 23 Tetrad of pollen grains showing differentiated nuclei, a generative nucleus (darker stained) and a vegetative nucleus (lighter stained) (1650X).

Figure 24 Tetrad of pollen grains with one microcyte (1650X).



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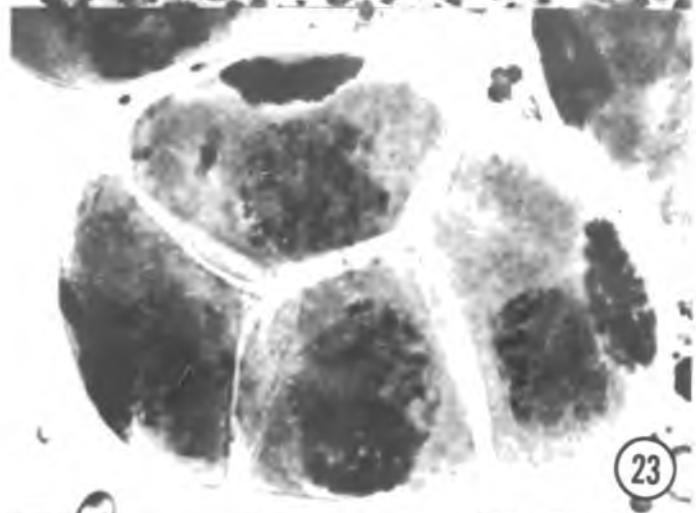

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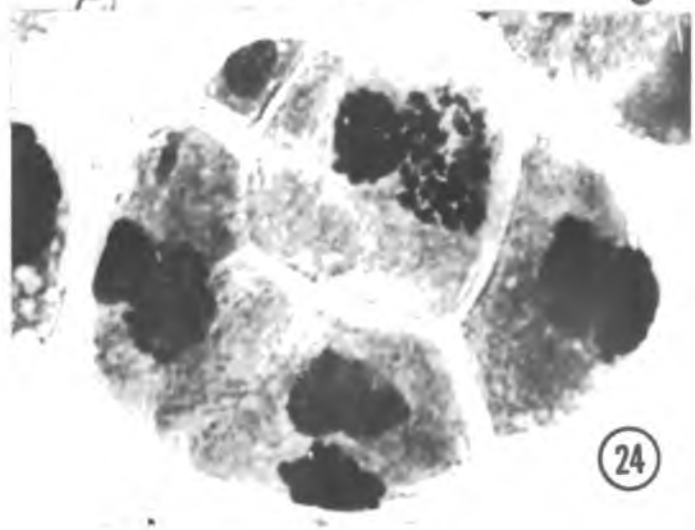

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Table 8. -- Sporad formation in tetraploid Aranda Wendy Scott 'Greenfield'

	Tetrad	Tetrad + 1 mc	Tetrad + 2 mc	Tetrad + 3 mc	Triad	Total No. of Sporads
No. of Sporads	247	93	53	6	1	400
%	61.8	23.3	13.2	1.5	0.2	100.0

Table 9. --Crosses with diploid and tetraploid *Aranda* Wendy Scott 'Greenfied' as an indication of fertility and sterility.

Cross			Pod	% Viable Seed
2N *Aranda* Wendy Scott	X	self	aborted	
	X	4N *Aranda* Wendy Scott	aborted	
	X	2N *Vanda* Miss Joaquim	aborted	
	X	4N *Vanda* Miss Joaquim	aborted	
	X	2N *Vanda lamellata*	formed	0% - no germ.
	X	2N *Vanda sanderiana*	aborted	
4N *Aranda* Wendy Scott	X	self	aborted	
	X	2N *Aranda* Wendy Scott	aborted	
	X	2N *Vanda* Miss Joaquim	aborted	
	X	4N *Vanda* Miss Joaquim	aborted	
	X	2N *Vanda lamellata*	formed	82% - good germ.
	X	2N *Vanda sanderiana*	formed	84% - good germ.

aborted except for the cross with Vanda lamellata. However, seed viability of this cross was 0%; thus no germination of the seeds occurred. On the other hand, the pods from the crosses between tetraploid Aranda Wendy Scott and V. lamellata and V. sanderiana did not abort, and seed viability was 82% and 84%, respectively. Both crosses had good germination of seeds.

V. Discussion

The differences in the frequencies of diploid and tetraploid plants of Aranda Wendy Scott in the 2 groups of mericlones might be attributed to the stage in meristem culture at which the initial mutation occurred, or the degree of admixture of diploid and tetraploid cells in the material used for sub-culture. The percentage of tetraploids in either group as well as in the total population (36.4%) is extremely high for a population of mericlones.

Wimber and Wimber (1968) found that tetraploids generally show an improvement in flower shape compared to the diploid, but plant growth and productivity is reduced. Based on a modification of Leonhardt's (1977) formula for flower shape, the difference in floral morphology between diploid and tetraploid Aranda Wendy Scott flowers was insignificant. According to Vajrabhaya (1977), when orchids are grown for cut flower purposes, the number of flowers per spray is of major importance, and diploids have a greater degree of productivity than tetraploids. Diploid Aranda Wendy Scott 'Greenfield' was found to be more vigorous and floriferous than the tetraploid. Diploid flower sprays were longer and contained more flowers per spray. Tetraploid flowers, on the other hand, were slightly larger than diploid flowers, and the vase life of the sprays were slightly longer in Group I. The differences did not appear in Group II probably due to the low number of tetraploid plants which produced a total of only 5 sprays over a period of 16 months. The data in Tables 3 and 5, Figures 2 and 4 for the tetraploids are based only on these 5 sprays, and therefore, the data in Group II were inadequate to measure differences.

For commercial cut flower purposes, spray yield, length of sprays, and number of flowers per spray are important factors. The high percentage of tetraploid mutants (36.4%) arising from mericloning diploid Aranda Wendy Scott 'Greenfield' must be considered undesirable for commercial cut flower purposes for this orchid cultivar.

Tanaka and Kamemoto (1961) found only univalent formation of the meiotic chromosomes in the hybrid Arachnis hookeriana X Vanda suavis. They concluded that these genera are distantly related due to the low homology of the genomes. They also found pollinia with dyads and tetrads with or without microcytes. Investigations of diploid Aranda Wendy Scott (Vanda Rothschildiana X Arachnis hookeriana) also revealed an irregular meiosis with a range of 3 to 16 bivalents and a low level of homology. However, compared to the Arachnis hookeriana X Vanda suavis cross, diploid Aranda Wendy Scott had a higher degree of meiotic irregularity, as shown by the wide range of number of bivalents and the types of sporads formed (dyads to nonads with or without microcytes).

Tetraploid Aranda Wendy Scott arose from spontaneous doubling of diploid somatic tissue during the process of meristem culture of vegetative propagation. Since the doubling was of somatic origin, then there should be 2 sets of identical chromosomes. Therefore, chromosome homology would be greatly increased, thereby increasing fertility (Stebbins, 1971). This increase in fertility is a result of the regularity of meiotic behavior. The majority of the sporads were tetrads with or without microcytes.

The crosses shown in Table 9 gives an indication of the fertility of both diploid and tetraploid forms of Aranda Wendy Scott. No progeny

was obtained from selfing or crossing diploid and tetraploid Aranda Wendy Scott or crossing diploid and tetraploid Aranda Wendy Scott with diploid and tetraploid Vanda Miss Joaquim. On the other hand, crossing tetraploid Aranda Wendy Scott with V. lamellata and V. sanderiana produced abundant viable seeds indicating the restoration of fertility in the tetraploid Aranda Wendy Scott. The failure of the cross between tetraploid Aranda Wendy Scott and V. Miss Joaquim might be attributed to an incompatibility system which is common in vandaceous orchids.

The restoration of fertility in Aranda Wendy Scott through chromosome doubling in meristem culture can be valuable for breeding purposes. The doubling of chromosomes in diploid Dendrobium Jaquelyn Thomas (Kamemoto, et al., 1964) restores regularity in meiotic behavior in the amphidiploid. Thus, although the tetraploid mutations of Aranda Wendy Scott 'Greenfield' arising in meristem culture are undesirable for commercial cut flower production, these mutants might circumvent the sterility barrier of this intergeneric hybrid and be valuable breeding material.

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